Warm autoadsorption with enzyme-treated red blood cells

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Patients demonstrating warm autoantibody specificity present serologic challenges for laboratory staff performing antibody identification in the blood bank. Autoantibody can be removed from plasma or serum by adsorption onto autologous red blood cells (RBCs) provided the patient has not been transfused in the previous 3 months. The adsorption process can be enhanced by enzyme pretreatment of autologous RBCs. ***Immunohematology* 2012;28:88–90.**

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Principle

Performing pretransfusion testing in the presence of warm autoantibodies optimally reactive at 37°C presents serologic challenges when attempting to determine the presence of underlying clinically significant alloantibodies.[1] Warm-reactive autoantibodies may mask the presence of other antibodies because of their agglutination with all cells tested, including autologous red blood cells (RBCs). To determine the presence of clinically significant antibodies, the removal of autoantibody may be achieved by adsorption of patient plasma or serum with autologous RBCs.[2–4] Allogeneic RBCs may be used for adsorption if the patient has been recently transfused or when the quantity of autologous RBCs is insufficient for autoadsorption.[2]

Indications

As circulating autologous RBCs are coated with autoantibody, antigen sites on the autologous cells may become blocked with immunoglobulin. To efficiently achieve autologous adsorption of warm-reactive autoantibodies, autoantibody must be dissociated from the RBC before the adsorption process.[2] Autoantibody dissociation can be accomplished by several methods, including partial heat elution at 45°C, gentle heat elution at 56°C, a combination of 0.2 M dithiothreitol (DTT) and papain or ficin treatment, acid-EDTA treatment, ZZAP (a mixture of cysteine-activated proteolytic papain and DTT) treatment, and chloroquine diphosphate (CDP) treatment.[5] (For specific procedures concerning these methods and reagents, see the applicable procedural reference documents or manufacturer's package insert.) After dissociation of the autoantibody from the autologous RBCs, the antigen sites should be available for binding the autoantibody from the plasma or serum during the adsorption process.

To further enhance the adsorption process, autologous RBCs can be treated with proteolytic enzymes such as ficin or papain. This additional treatment enhances antibody uptake onto the autologous cells by cleaving some of the glycoprotein chains extending from the cell membrane, leading to improved accessibility of the antigens not removed by the dissociation method.[4]

Reagents/Supplies

Enzyme Treatment Method	Reagents		Supplies
Papain	1% cysteine-activated papain	▪ Isotonic saline ▪ Patient autologous red blood cells	▪ 1 mL graduated pipettes ▪ 37°C water bath ▪ Large bore test tubes ▪ Calibrated centrifuge
Ficin	1% ficin		

Procedural Steps

Treatment of Cells	▪ Mix enzyme with packed red cells ▪ Incubate mixture ▪ Wash mixture to remove enzyme ▪ Centrifuge to pack the red cells ▪ Remove supernatant
Adsorption	▪ Mix enzyme-treated cells with patient plasma/serum ▪ Incubate the mixture ▪ Centrifuge the mixture ▪ Remove plasma/serum

Procedure

For papain or ficin (1%) treatment of RBCs, one volume of 1% cysteine-activated papain or 1% ficin is added to two volumes of packed RBCs. For example, if 2 mL of packed RBCs is treated, 1 mL of ficin or papain is added. The mixture

is incubated at 37°C for a predetermined time to allow for the most effective enzyme treatment. If commercially prepared enzyme is used, the manufacturer's insert is used to determine the incubation time. If enzyme is prepared in-house, qualification studies are performed to determine the appropriate treatment time.[6]

The enzyme-treated RBCs are washed with copious amounts of isotonic saline a minimum of three times, or until the supernatant saline is clear, to ensure complete removal of enzyme. The last wash is centrifuged a minimum of 5 minutes at 900 to 1000× *g* to completely pack the RBCs.[7] This step is critical to achieve proper packing of RBCs and removal of maximum amounts of saline. To avoid plasma or serum dilution during adsorption, as much saline as possible is carefully removed after the final wash. This can be accomplished with suction using a small-bore pipette or filter paper.[4]

To perform adsorption, one volume of enzyme-treated RBCs is mixed with an equal volume of the patient's plasma or serum. To enhance antibody uptake, the proportion of RBCs to plasma or serum can be increased. Adsorption is more effective if the area of contact between the RBCs and plasma or serum is large; use of a large-bore test tube (16 × 100 mm) increases the surface area and allows for optimum plasma or serum and RBC contact. The mixture is incubated at 37°C for 10 to 60 minutes.[4] The mixture is then centrifuged and the plasma or serum is carefully removed. If the harvested adsorbed plasma or serum will be used for additional adsorptions, disturbing the packed RBCs to achieve maximum plasma removal is acceptable. If, however, the harvested adsorbed plasma or serum will be used for antibody detection, the packed RBCs should not be disturbed in an attempt to recover additional plasma.

The number of adsorptions required to exhaust autoantibody from the plasma or serum is proportionally related to the strength of its reactivity in plasma or serum; however, in some instances, additional adsorptions may be required to remove the antibody.[7–9] If the original plasma or serum reactivity is 1+ with the indirect antiglobulin test (IAT), the first aliquot of adsorbed plasma or serum should be tested against group O reagent RBCs or direct antiglobulin test (DAT) negative autologous RBCs, if available. If reactivity persists, the adsorption procedure should be repeated, as needed, using a fresh volume of enzyme-treated RBCs. Once reactivity is abolished, the adsorbed plasma or serum is tested against the appropriate group O reagent panel RBCs to exclude underlying alloantibodies.

Limitations

A recently transfused patient (within the last 3 months) is not a candidate for autologous adsorption. Circulating transfused RBCs may adsorb clinically significant plasma or serum alloantibodies.[2] It has been demonstrated that very small amounts of antigen-positive RBC are capable of eliminating alloantibody reactivity from the plasma or serum.[2,10]

Large volumes of autologous RBCs from the patient are needed to perform autoadsorption. In the case of a low patient hematocrit, it may be difficult to obtain a sufficient volume of autologous cells for testing without exacerbating clinical symptoms of anemia.

Cell treatment may be unsuccessful in stripping autoantibody from the autologous, coated RBCs. As a result, adsorption may be less efficient in removing autoantibody.

Enzyme treatment of RBCs will destroy antigen sites on the RBCs. Enzymes remove sialic acid from the RBCs and will destroy some antigens (-M, -N, -S, -s, -Fy^a, -Fy^b, -En^a, -Ge, -JMH, -Ch/Rg, -In^b). Autoantibodies specific for enzyme-sensitive antigens will not be removed by this method.[4]

Potential dilution of adsorbed plasma or serum must be considered a consequence of preparation of enzyme-treated cells. If the saline is not properly removed after the final wash, the residual saline will dilute the plasma or serum when adsorptions are performed. The more adsorptions performed, the greater the dilution factor. As a result, diluted antibody may become undetectable in the adsorbed plasma or serum.

Enzyme treatment of autologous RBCs can cause hemolysis. If the RBCs are hemolyzed by enzyme treatment, there may be an insufficient quantity of RBCs remaining to perform adsorptions.

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References

1. Leger RM, Garratty G. Evaluation of methods for detecting alloantibodies underlying warm autoantibodies. Transfusion 1999; 39:11–16.
2. Leger, RM. Positive DAT and immune-mediated hemolysis. In: Roback JD (ed). Technical manual. 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:505–6.

3. Morel PA, Bergren MO, Frank BA. A simple method for the detection of alloantibody in the presence of autoantibody [abstract]. Transfusion 1978;18:388.
4. Judd J, Johnson ST, Storry JR. Judd's methods in immunohematology. 3rd ed. Bethesda, MD: American Association of Blood Banks, 2008:45, 457–9.
5. Beattie K, Crawford M, Martin, J, et al. Immunohematology methods. Rockville, MD: American Red Cross, 1993:44-1, 48-1, 51-1.
6. Method 3-5-1: Preparing ficin enzyme stock, 1% W/V; Method 3-5-2: Preparing papain enzyme stock, 1% W/V; Method 3-5-3: Standardizing enzyme procedures. In: Roback JD (ed). Technical manual. 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:902–3.
7. Method 4-8: Adsorbing warm-reactive autoantibodies using autologous red cells. In: Roback JD (ed). Technical manual. 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:924–5.
8. Garratty G. What do you do when all units are incompatible? 43. Instituto Mexicano del Seguro Social, 2005:S107–11.
9. Method 3-12: Adsorption procedure. In: Roback JD (ed). Technical manual. 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:914.
10. Garratty G, Telen MJ, Petz LD. Red cell antigens as functional molecules and obstacles to transfusion. Hematology AmSoc Hematol Educ Program 2002:445–50.

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